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Effect of Immediate Application of Pomegranate Peel, Grape Seed and Green Tea Extracts on Composite Shear Bond Strength of In-Office Bleached Enamel

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Abstract: This *in vitro* study evaluated the effectiveness of 5% solution of grape seed, pomegranate peel, green tea extracts and a 10% solution of sodium ascorbate on composite shear bond strength of bleached enamel. The 70 bovine incisors were divided in 7 groups (n = 10); group A: Immediate bonding; B: 2 weeks delay bonding; C: 10% sodium ascorbate; D: 5% green tea extract; E: 5% pomegranate extract; F: 5% grape seed extract; control: No bleaching, no antioxidant. For groups A-F, hydrogen peroxide gel was applied on the labial surface of teeth then composite with dimension of 5 mm diameter and 2 mm height was placed. Universal testing machine measured the shear bond strength. The data was analyzed using one-way ANOVA and Tukey tests ($p \leq 0.05$). Group A exhibited the lowest shear bond strength among the groups (statistically significant; $p<0.0001$). There was no significant difference between group B-F and control ($p>0.05$). All the antioxidants used in this study was equally effective to neutralize adverse effects of hydrogen peroxide on composite bond strength of enamel immediately after bleaching.

Key words: Antioxidant, shear bond strength, tooth bleaching, grape, seed, green tea

INTRODUCTION

Esthetic of anterior teeth is an important approach in the modern dentistry (Khoroushi and Aghelinejad, 2011). Bleaching has been used to achieve better appearance of teeth for more than a century (Summitt *et al*., 2006).

Hydrogen peroxide or its derivatives are used in various concentration in most bleaching techniques (Heymann *et al*., 2012). The use of dental bleaching agents has various side effects, such as pulpal sensitivity, changes in the tooth structure, microleakage in existing restorations, external root resorption (Khoroushi and Saneie, 2012; Sharafeddin and Varachehre, 2008; Sharafeddin and Jamalipour, 2010). Hydrogen peroxide also has an adverse effect on bond strength of composite resin to etched enamel when bonding agent is applied immediately after the bleaching process (Vidhya *et al*., 2011).

The widely accepted explanation refers to the function of bleaching agents, hydrogen peroxide is able to diffuse in organic matrix of enamel and dentin due to its low molecular weight (Summitt *et al*., 2006) with the disintegration free radicals, such as hydroxyl radicals, superoxide anions, nascent oxygen and perhydroxyl form these free radicals can react with unsaturated bonds and break down the large organic molecules (Vidhya *et al*., 2011).

Presence of these free radicals interfere with the formation of resin tags and adhesive polymerization (Khoroushi and Aghelinejad, 2011; Lima *et al*., 2011; Vidhya *et al*., 2011). However after a reasonable time, these residual oxygen gradually disperse and resin is able to bond to the bleached enamel favorably (Muraguchi *et al*., 2007). Adequate time interval between bleaching and bonding process is reported from 24 h to 4 weeks (Heymann *et al*., 2012; Summitt *et al*., 2006; Vidhya *et al*., 2011).

A variety of methods have been proposed to overcome this problem immediately after bleaching, including the removal of the superficial enamel, treatment of the bleached enamel with alcohol before bonding procedure, application of acetone-based adhesives and using an antioxidant agent such as a gel or solution of 10% sodium ascorbate and 10% solution of alphatocopherol (Khoroushi and Aghelinejad, 2011; Dabas *et al*., 2011; Muraguchi *et al*., 2007; Vidhya *et al*., 2011).

One study compared the effect of two different sodium ascorbate concentrations (10 vs. 20%) on the shear bond strengths of resin to bleached enamel and stated that both concentrations result in significant greater bond strength than the bleached group and were not significantly different from each other (Kimiayi and Valizadeh, 2006).

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In a recent study, it has been reported that grape seed extract, a natural antioxidant, neutralize the harmful effects of the bleaching agent on bond strength of bleached enamel (Vidhya *et al*., 2011). One may assume that other antioxidants used in medical field may have similar effect with grape seed extract.

The dry leaves of green tea (*Camellia sinensis*) contain flavonols with antioxidant capacity and have a protective effect against coronary heart diseases and some cancers caused by chemical carcinogens (Bornhoeft *et al*., 2012; Nederkassel *et al*., 2005; Yokozawa *et al*., 2012). Pomegranate peel extract contains phenolic compounds which have anti mutagenic and carcinogenic capacities and it can reduce the risk of cancers particularly prostate cancer (Ismail *et al*., 2012; Salgado *et al*., 2012; Neyrinck *et al*., 2012). It should be noted that these materials are dietary supplements so obviously they can be used clinically (Ismail *et al*., 2012; Nederkassel *et al*., 2005; Vidhya *et al*., 2011).

Since, few or no studies regarding the effect of these natural antioxidants on shear bond strength of bleached enamel have been found in literatures, the aim of this study is to evaluate and compare the effectiveness of 5% solution of grape seed, pomegranate peel, green tea extracts and a 10% solution of sodium ascorbate on shear bond strength of bleached enamel.

MATERIALS AND METHODS

In this experimental study, the 70 extracted intact incisors were stored in 0.1% thymol solution at room temperature. The roots were embedded in the fabricated acrylic resin blocks from CEJ so that the coronal portions of the teeth were out of the blocks.

Labial surface of the teeth were flattened with 600 grit aluminum oxide abrasive paper in low speed handpiece with constant water coolant. The teeth were divided into 7 groups of 10 teeth each group (group A-F):

- Group A; composite restoration was performed immediately after bleaching
- Group B; after bleaching treatment, teeth were stored in distilled water at room temperature for 2 weeks before composite restoration
- Group C; to prepare 10% solution of sodium ascorbate, 10 g of sodium ascorbate (AppliChem, Darmstadt, Germany) was dissolved in 100 mL distilled water and was applied on the labial surface of the teeth for 10 min immediately after bleaching, then rinsed off and composite restoration was performed
- Group D; to prepare a solution of 5% green tea extract, first 5 g of dried green tea leaves extract (CAMGREEN, G.E.P.co, Iran, Gorgan) was dissolved in 100 mL distilled water then was applied on the labial surface of teeth for 10 min immediately after bleaching then rinsed off and composite restoration was performed
- Group E; to prepare a solution of 5% pomegranate peel extract (Anar, Amin Pharmaceutical Co, Iran, Isfehan) 5 g of dried pomegranate peel extract was dissolved in 100 mL distilled water then was applied on the labial surface of teeth for 10 min immediately after bleaching then rinsed off and composite restoration was performed
- Group F; to prepare a solution of 5% grape seed extract (Puritans Pride Inc, Oakdale, NY, USA), 5 g of grape seed extract was dissolved in 100 mL distilled water then was applied on the labial surface of tooth for 10 min immediately after bleaching then rinsed with water and composite restoration was performed
- Control group; no bleaching was performed, teeth were restored with composite

For those groups which received bleaching treatment (A-F), a 38% hydrogen peroxide gel (Opalacence Xtra Boost, Ultradent products Inc, South Jordan, UT) was applied on the labial surface of teeth for 20 min according to the manufacturer instruction. The gel was rinsed off thoroughly with tap water and the process was repeated one more time.

The restoration process was the same for all groups. The labial surface of the teeth was etched with 35% phosphoric acid for 15 sec (3M ESPE, St Paul, MN, USA), rinsed with water for 15 sec and thoroughly dried to see frosted appearance. A bonding agent (adeper single bond, 3 M ESPE, USA) was applied in two consecutive layers with gentle air evaporation between the layers and were light-activated for 20 sec. Then a composite build up (Filtek Z 100, 3M ESPE, USA, shade A3) was placed on the bonded surface (5 mm diameter and 2 mm height) and was light activated for 40 min with LED light curing unit.

All the samples were stored in distilled water at room temperature for 24 h before test. To measure the shear bond strength, samples were placed in the universal testing machine (Zwick roell testing machine Pvt. Ltd., Germany). The load was applied at the junction of the composite-enamel with 0.5 mm min^{-1} crosshead speed. The data were statistically analyzed by adapting the SPEE Statistical Software and using one-way ANOVA and Tukey Test at the significant level of 0.05.

RESULTS AND DISCUSSION

The mean value and standard deviation for each group are listed in Table 1 and illustrated in Fig. 1. The one-way ANOVA revealed significant difference among groups (p<001). The Tukey test showed that only the means of the group A exhibited the significant lowest shear bond strength among the 7 groups of present study. However, there was no significant difference between group B-F and control (Table 2).

The results of this study indicate that immediate applying of bonging agent to the bleached enamel results in significant reduction in the composite shear bond strength. These results are consistent with the most previous studies (Heymann *et al*., 2012; Khoroushi and Aghelinejad, 2011; Mazaheri *et al*., 2011; Sunfeld *et al*., 2005; Vidhya *et al*., 2011). Using optical polarized light microscopy one study showed that extension of resin tags compromised when bonding process performed immediately after bleaching treatment (Sunfeld *et al*., 2005).

Table 1: Mean and standard deviation of shear bond strength of each groups (Mpa)

Groups	Mean±SD	p-value*
A	15.49±5.56	<0.001
B	26.24±5.56	
C	25.83±3.05	
D	27.25±4.06	
E	27.21±5.40	
F	24.10±5.03	
Control	25.44±4.58	

*Using one-way ANOVA; Group A = Immediate bonding; Group B = Delay bonding; Group C = 10% sodium ascorbate; Group D = 5% green tea extract; Group E = 5% pomegranate extract; Group F = 5% grape seed extract; Group Control = no bleaching, no antioxidant

Table 2: Muliple comparisons of significancy (p value) of groups*

Groups	B	C	D	E	F	Control
A	0.001	0.001	0.000	0.000	0.013	0.002
B	-	1.000	1.000	1.000	0.974	1.000
C	1.000	-	0.997	0.997	0.992	1.000
D	1.000	0.997	-	1.000	0.853	0.989
E	1.000	0.997	1.000	-	0.859	0.990
F	0.974	0.992	0.853	0.859	-	0.998

* = Using Tukey test; Group A = Immediate bonding; Group B = Delay bonding; Group C = 10% sodium ascorbate; Group D = 5% green tea extract; Group E = 5% pomegranate extract; Group F = 5% grape seed extract; Group control = no bleaching, no antioxidant

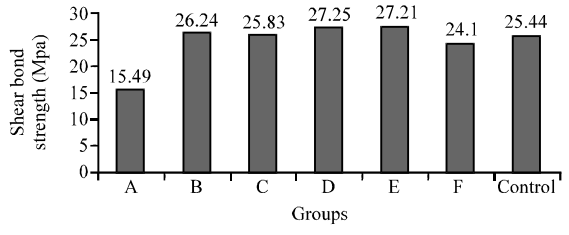

Fig. 1: Mean shear bond strength (Mpa) between different groups; A = Immediate bonding; B = Delay bonding; C = 10% sodium ascorbate; D = 5% green tea extract; E = 5% pomegranate extract; F = 5% grape seed extract; Control = No bleaching, no antioxidant

A SEM examination of interface between bleached enamel and resin suggested presence of defective resin tags which penetrate lesser depth than unbleached enamel (Mazaheri *et al*., 2011). It has been shown that oxygen released from hydrogen peroxide is trapped during light polymerization and may lead to bubble formation in the adhesive layer (Torres *et al*., 2006).

The data from this study revealed that lag in the bonding process for 2 weeks does neutralize diverse effects of the bleaching agents on bond strength of bleached enamel as group B did not have significant difference with group control (p>0.05). It was similar to some previous studies (Khoroushi and Aghelinejad, 2011; Heymann *et al*., 2012; Summitt *et al*., 2006; Sunfeld *et al*., 2005; Vidhya *et al*., 2011). The explanation may be that immersion in water causes dispersion of residual oxygen remains in enamel matrix (Mazaheri *et al*., 2011).

Ascorbic acid and its salt, sodium ascorbate are reducing agents that are used in the food industry and are not toxic and seems unlikely to have systemic or local harmful effects (Summitt *et al*., 2006; Torres *et al*., 2006). Ascorbic acid in hydro-gel or solution forms have been used with application times of 10 min to one third of the application time of bleaching agents (Vidhya *et al*., 2011). Since, ascorbic acid is a week acid, it is recommended to use sodium ascorbate rather than the ascorbic acid to avoid unintended double etching effect (Torres *et al*., 2006). The current study used 10% solution of sodium ascorbate for 10 min immediately after bleaching treatment which led to increase bond strength same as in the control group and in the group B with 2 weeks storage before bonding process. This results are similar to previously reported findings that introduced effective application of Sodium ascorbate for the same purpose (Summitt *et al*., 2006; Mazaheri *et al*., 2011; Torres *et al*., 2006; Vidhya *et al*., 2011).

Proanticyanidin is a natural plant metabolite. It is a mixture of monomers, oligomers and polymers of catechins which found in fruits seeds. It is a potent natural antioxidant and free radical scavenger which in medical field is known as an anti allergen, bacterial and carcinogenic compound with proved vasodilator properties (Summitt *et al*., 2006; Xie *et al*., 2008). Grape seed extract contains 98% proanthocyanidine that has gained popularity as an dietary supplement (Summitt *et al*., 2006; Xie *et al*., 2008). It seems

that grape seed extract inhibit demineralization and enhance re-mineralization of *in vitro* root caries (Xie *et al*., 2008).

A recent study concluded that treatment with 5% solution of grape seed extract increases the bond strength of enamel compared with bleached and unbleached enamel (Vidhya *et al*., 2011). Present study showed that the use of grape seed extract provides stronger bond compared with immediate bonded group, however the bond strength did not exceed the unbleached control group.

Green tea is made from leaves of *Camelia sinensis* and is known as a popular beverage around the world (Bornhoeft *et al*., 2012). Several studies have shown that green tea polyphenols have free radical scavenging ability (Bornhoeft *et al*., 2012; Nederkassel *et al*., 2005; Yokozawa *et al*., 2012). This antioxidant activity is associated with protective effect against diseases caused by oxidative stress including renal disorders, coronary heart disease, cancers (lung, esophagus, liver, breast, etc.) (Bornhoeft *et al*., 2012; Nederkassel *et al*., 2005; Yokozawa *et al*., 2012). This study confirms the effectiveness of this compound against hydroxyl radical and superoxide anion. Group D that 10% solution of green tea extract was used immediately after bleaching for 10 min before bonding process researchers observed higher bond strength values than group A and comparable to control group which means that application of this solution have been successful in reversing reduced bond strength. During this study, researchers observed superficial staining after application of this solution which was removed in subsequent ethching process.

In the recent years, there is much attention to the nutritional benefits of pomegranate (*Punica granatum*) (Bornhoeft *et al*., 2012). It has high antioxidant activity due to the high content of phenolic compounds, especially elagic acidIt is claimed that the fruit peel has a higher polyphenol level than the seeds and pulps (Bornhoeft *et al*., 2012; Salgado *et al*., 2012). In traditional medicine it has been used to treat diabetes, diarrhea and inflammatory disease for centuries (Ismail *et al*., 2012; Neyrinck *et al*., 2012). From the toxological point of view, pomegranate peel extract is safe and non-toxic (Ismail *et al*., 2012). In this study, researchers used solution of pomegranate peel extract (5%) to neutralize the negative effects of free radicals on the bonding agents polymerization. It was shown to be effective, since there were no significant differences between the group E and the control group.

There was no significant differences between the groups treated with antioxidants (group C-F) and the group B which indicates that treatment with these antioxidants is an effective and time-efficient method. Moreover, there was no significant differences among antioxidant groups (C-F) and it seems that all the solutions used in the current study were equally effective. Therefore, various antioxidants based on their availability can be used to increase the bond strength of composite restorations immediately after in-office bleaching procedure. Antioxidant effects on bond strength after at-home bleaching is the question that can be answered in subsequent investigations.

CONCLUSION

This study showed that treatment with 38% hydrogen peroxide bleaching gel decreases the bond strength of adhesive resin to enamel if the bonding process carries on immediately after bleaching. A delay of 2 weeks after bleaching results in increased bond strength of bleached enamel compared with the immediate application of bonding agent to bleached enamel.

Immediate application of 10% solution of sodium ascorbate and 5% solution of grape seed extract, green tea extract and pomegranate peel extract for 10 min restores the bond strength to the basic strength. All the antioxidants used in this study (sodium ascorbate, grape seed extract, pomegranate peel extract, green tea extract) are equally effective to neutralize the free radical adverse effects on composite bond strength of enamel.

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